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Effects of prenatal and lactation nicotine exposure on glucose homeostasis, lipogenesis and lipid metabolic profiles in mothers and offspring

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There is increasing evidence suggesting that maternal nicotine (NIC) exposure alone can lead to many deleterious consequences in the fetus. In this study, we aimed to evaluate the effects of prenatal and lactation NIC exposure on glucose homeostasis, lipogenesis and lipid metabolism in mothers and pups. After maternal NIC exposure (from gestational day 9 to weaning), NIC mothers showed lower body weight, decreased parametrial white adipose tissue (pWAT) and inguinal WAT weights, lower homeostasis model assessment of beta cell function, higher serum total cholesterol (TC) and low-density lipoprotein cholesterol levels, higher Castelli index values, lower hepatic mRNA levels of sterol regulatory element binding protein-1c (SREBP1c), lipoprotein lipase, acetyl-CoA carboxylase, fatty acid synthase (FAS) and glucose transporters 4 (GLUT4), as well as lower SREBP1c, FAS, leptin and GLUT4 mRNA levels in pWAT. However, female NIC pups presented higher body weights and serum TC levels, and increased trends for high density lipoprotein-cholesterol and Castelli index I. Male NIC pups had higher body weight, serum TC levels and Castelli index I values, and lower glycemia levels. Additionally, hepatic and adipose FAS gene expression from the female NIC pups presented a decreasing trend, while the male NIC pups had lower hepatic FAS expression and higher adipose FAS expression. In conclusion, prenatal and lactation NIC exposure induced deleterious effects on the glucose homeostasis, lipogenesis and lipid metabolism in both mothers and pups, which may promote several important metabolic disorders in the progeny. Additionally, there are gender-specific effects on pups.

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1 Introduction

Although maternal smoking is associated with adverse fetal, obstetric and developmental outcomes,^{1–5} approximately 9%–20% of mothers worldwide continue to smoke throughout their entire pregnancy.^{6–8} Additionally, this number increased during the breast-feeding period because many mothers returned to smoking after delivery.⁹ Recently, a meta-analysis study has shown that babies whose mothers smoked regularly during pregnancy had a 47% increase in the odds of becoming overweight.¹⁰ Moreover, Ma *et al.* suggested that it was the direct and long-term effects of intrauterine exposure to the chemicals in cigarette smoke that accounted for the increased risk of obesity in offspring whose mother smoked during pregnancy.¹¹ Among all of the chemicals constituting cigarette

smoke, nicotine is considered to be one of the major adverse components that perturbs fetal development.¹² Animal studies have recently demonstrated that nicotine might be the single most important component of cigarette smoke leading to long-term adverse metabolic outcomes, including obesity.^{13–16} Nicotine can easily pass through membrane barriers and activate nicotinic acetylcholine receptors.^{17,18} Therefore, nicotine can distribute in placental tissue, amniotic fluid, fetal blood and breast milk,^{19–22} leading to significant fetal and neonatal exposure. However, maternal exposure to nicotine during gestation and lactation is not restricted to cigarette smoking alone. Several nicotine-based pharmacotherapies for smoking cessation have been developed (*e.g.*, e-cigarettes and nicotine replacement therapy), and their usage has been considered to be of benefit for pregnant women with heavy nicotine dependence and they have become more and more popular in recent years, especially amongst adults of reproductive age.^{23–25} The effects of maternal exposure to nicotine alone have been long overlooked when compared with the deleterious health risks of cigarette smoking; however, previous studies were not sufficient to confirm the safety and efficacy of using these

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nicotine-containing products during pregnancy and lactation.^{26–28} In fact, increasing evidence suggests that maternal nicotine exposure alone can lead to many deleterious consequences in the fetus; therefore, a more comprehensive evaluation of the short- and long-term health effects of nicotine on the offspring is necessary.²⁹

Several epidemiological and animal studies have shown that environmental changes within a critical window of development, such as gestation or lactation, can initiate permanent alterations in metabolism that lead to diseases in adulthood. This association has been called programming, which may play an important role in the development of obesity.^{30–32} The placenta provides a link between the circulations of the mother and the fetus, which ensures proper fetal development through the regulation of nutrient and gas transfer from the mother to the fetus.³³ Epidemiological data have confirmed that maternal smoking during pregnancy might be a risk factor for childhood obesity.^{34–36} It was reported that rats exposed to nicotine *in utero* showed increased body weight, body fat and visceral adipose tissue at birth and at 9 weeks of age.^{37,38} Lactation is a critical period of life: important cognitive and neurological developments occur in this period. Mother's milk represents the primary source of nutrition in this period.³⁹ However, only a few studies have suggested that the first postnatal week is critical for nicotine programming of body weight and body fat distribution. Oliveira *et al.* identified that lactation is sensitive to the isolated effects of nicotine, and maternal nicotine exposure during lactation has short- and long-term effects on body weight regulation, body adiposity, leptin concentration, hormonal status and thyroid function in rat offspring.^{21,40} Studies have also shown that prenatal and lactation nicotine exposure affects the body weight, fat pad weight, and perivascular adipose tissue in offspring at 35 days, 70 days and 6 months of age.^{41,42} Therefore, it was established that maternal nicotine exposure is closely related to an obese offspring phenotype in adulthood. Recently, Santos-Silva *et al.* found that maternal tobacco exposure during lactation led to changes in nutritional, biochemical, and hormonal parameters in dams and these changes, passively through the milk, may promote several important metabolic disorders in the progeny.⁴³ Oliveira *et al.* also showed that maternal nicotine exposure during lactation resulted in important nutritional, biochemical, and hormonal parameter changes in mothers and their offspring.²¹ However, little is known about the effect of prenatal and lactation nicotine exposure on mothers and their offspring. Are the patterns of these effects clearly distinct from the effects of lactation nicotine exposure alone? Can these effects on the mother further affect the fetus or the newborn and then be important for the programming effects observed in adverse metabolic diseases?

Metabolic diseases are characterized by glucose and lipid metabolic disorders. Maternal metabolic abnormalities may affect the fetal metabolic state. Previously, we utilized NMR-based metabolomics to demonstrate that prenatal nicotine exposure caused glucose, lipid and protein metabolism changes in the maternal plasma, fetal plasma and amniotic

fluid.⁴⁴ To the best of our knowledge, only a small number of research studies have evaluated the effects of nicotine exposure on the glucose homeostasis, lipogenesis and lipid metabolic profiles of the mothers and pups during the gestational to the early postnatal period. Thus, this study was aimed to evaluate the short-term consequences of maternal nicotine exposure during gestation and lactation on the glucose homeostasis, lipogenesis and lipid metabolic profiles of mothers and pups. This study has important significance in elucidating the developmental origin of obesity caused by maternal nicotine exposure.

2 Materials and methods

2.1 Animals

Pathogen-free Wistar rats weighing 180–220 g (female) or 260–300 g (male) were obtained from the Experimental Center of Hubei Medical Scientific Academy (no. 2008-0005, Hubei, China). The rats were maintained under 12 h light : 12 h dark cycles, at 22–24 °C room temperature. The animals were allowed to acclimate for at least one week before being subjected to experimental conditions. Each male rat was mated with two female rats, and the occurrence date of the vaginal plug or sperm in the vaginal smear was considered gestational day (GD) 0. After mating, each pregnant rat was placed in an individual cage with free access to water and food until weaning (28 days of lactation).^{45,46} The animal studies were performed at the Wuhan University Center for Animal Experimentation (Wuhan, Hubei, China), which has been accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). The study protocol was designed in accordance with the Guidelines for Animal Research and was approved by the Medical College of Wuhan University Ethical and Research Committee.

2.2 Prenatal and lactation nicotine exposure

After mating, each pregnant rat was randomly assigned to a nicotine (NIC) or a control (C) group. Nicotine (Sigma, St Louis, MO, USA) was subcutaneously administered (1.0 mg kg^{−1}) to pregnant rats or dams twice per day from GD 9 to weaning,⁴⁷ and the control group was administered the same volume of vehicle. According to the previous study, this dose of nicotine could produce similar plasma nicotine levels with those observed in moderate to heavy smokers.^{48–51} In general, pregnant rats produced 12–16 pups. We only used litters in which the pups of each gender were no less than 6. All litters (NIC, *n* = 8; C, *n* = 8) were adjusted to 12 pups (6 males and 6 females) at postnatal day 1 (PND 1) so as to avoid the influence of the litter size. Before weaning, the dams and offspring were fasted for 12 hours and placed in a separate quiet room for anesthesia. After the disappearance of the righting reflex, the animals were killed by rapid decapitation. Blood, perirenal white adipose tissue, epididymal (eWAT) or parametrial white adipose tissue (pWAT), inguinal subcuta-

neous white adipose tissue (iWAT) and livers were collected. The adipose tissue and livers were immediately frozen in liquid nitrogen and then stored at -80°C for subsequent experiments.

2.3 Body weight and organ index

During the entire pregnancy and lactation period the mothers' body weights were monitored daily and the pups' body weights were monitored every week. Liver weights and adipose weights of the mothers and pups were measured at weaning.

2.4 Serum biochemical parameters

Blood samples were centrifuged (1500g, 10 min) to obtain serum, and they were kept at -20°C until they were assayed. Blood glucose, total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and non-esterified fatty acid (NEFA) levels were detected using commercial test kits with a Hitachi 7600 Series automatic biochemical analyzer (Hitachi, Tokyo, Japan).

2.5 Serum hormone quantification by ELISA

Serum insulin was determined with a commercial rat ELISA kit (Mercodia, Sweden) (the detection limit was $\leq 0.020\ \mu\text{g L}^{-1}$). Leptin was measured with a specific rat ELISA kit (Abcam, Inc., Cambridge, MA, USA) (sensitivity: $\geq 30\ \text{pg mL}^{-1}$; intraassay variation: $<10\%$).

2.6 Insulin sensitivity evaluation

Insulin sensitivity and beta cell function were analyzed according to the following two formulae:^{52,53}

- (1) homeostasis model assessment of insulin resistance $\text{HOMA-IR} = \text{Insulin (mIU/mL)} \times \text{serum glucose (mmol/L)} / 22.5$;
- (2) homeostasis model assessment of beta cell function $\text{HOMA-}\beta = [\text{Insulin (mIU/mL)} \times 20] / [\text{serum glucose (mmol/L)} - 3.5]$.

2.7 Castelli index I and II calculations

The Castelli I and II indices, which correlate with atherogenesis, were obtained using the following formulae:^{21,54}

- (1) Castelli index I = total cholesterol/HDL-C
- (2) Castelli index II = LDL-C/HDL-C

2.8 Gene expression involved in lipogenesis and metabolism

Total RNA was isolated from the liver and eWAT or pWAT using Trizol according to the manufacturer's protocol. The relative expressions for rat sterol regulatory element binding protein-1c (SREBP1c), lipoprotein lipase (LPL), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), leptin, adiponectin and glucose transporter 4 (GLUT4) were determined using an ABI Step One real-time PCR thermal cycler (ABI Stepone, NY, USA). We quantified the PCR products using a SYBR Green Core Reagent kit (Applied Biosystems) and calculated the relative gene expression levels with the $\Delta\Delta\text{C}_t$ method using β -actin (for liver) or acidic ribosomal phosphoprotein P0 (36B4) (for eWAT or pWAT), as an internal control. Primers were designed using Premier 5.0 and the sequences of the primers used are listed in Table 1.

2.9 Statistical analysis

Results were reported as $\bar{x} \pm \text{S.E.M.}$ Statistical analysis and graphics were performed using Prism (GraphPad Software, Inc., La Jolla, CA, USA; version 5.0). All results were evaluated using an unpaired Student's *t*-test, where a *P*-value of less than 0.05 was considered to be significant. We studied two offspring (one male and one female) from each mother at weaning. However, for the birth weight, the litter was used as the experimental unit; therefore, we considered the average values from the same gender of the same litter instead of using individual animal values.

3 Results

3.1 Effects of prenatal and lactation nicotine exposure on mothers

3.1.1 Body weight and organ index. Before nicotine exposure, there were no differences in the mothers' body weight between the NIC and C groups. However, compared with the control, the NIC mothers showed lower body weights from GD 15 to the end of lactation ($P < 0.01$, Fig. 1A and B), and in particular, there was a 6% and a 10% decrease at the end of the pregnancy and lactation periods, respectively ($P < 0.01$, Fig. 1A and B). At weaning, the NIC mothers also exhibited lower pWAT, iWAT and liver weights (-53% , -23% and -18% , Fig. 1C, $P < 0.05$, $P < 0.01$, respectively). Additionally, the pWAT

Table 1 Real-time PCR primers and conditions

Genes	Forward primer	Reverse primer	Product (bp)
SREBP1c	AGGGAGTTCCTCAGATGCTCTTG	CATGCTGGAAGTACAGAGAAG	99
LPL	TCTCCTGATGATGCGGATTT	CAACATGCCCTACTGGTTTC	97
ACC	GGACCACTGCATGGAATGTAA	TGAGCGACTGCCGAAACATCTC	133
FAS	AGGATGTCAACAAGCCCAAG	ACAGAGGAGAAGGCCACAAA	100
Leptin	TTTCACACACGACGTCGGTATC	GGTCTGGTCCATCTTGACAAA	101
GLUT4	CTTGATGACGGTGGCTCTGC	CACAATGAACCAGGGGATGG	127
β -Actin	ATGGATGACGATATCGCTGC	CTTCTGACCCATACCCACCA	150
36B4	CGACCTGGAAGTCCAACCTAC	ATCTGCTGCATCTGCTTG	109

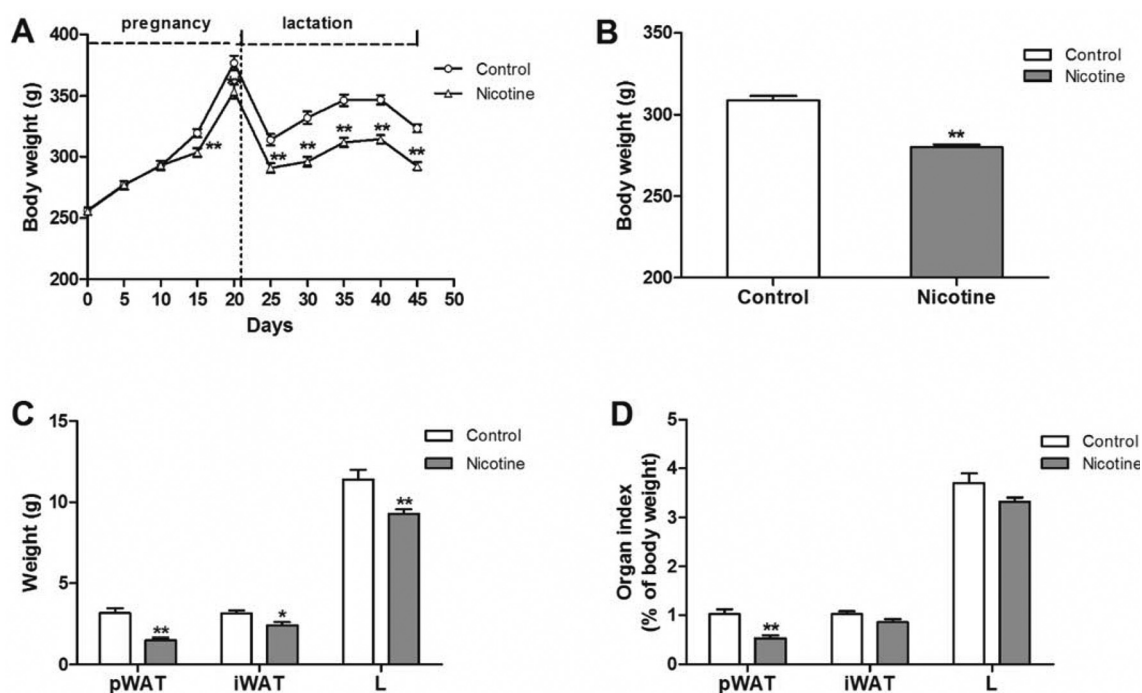


Fig. 1 Total body weight, parametrial white adipose tissue (pWAT), inguinal subcutaneous white adipose tissue (iWAT) and liver (L) weights of nicotine-exposed mothers during gestation and lactation ($\bar{x} \pm \text{S.E.M.}$, $n = 8$). (A) Body weight during gestation and lactation. (B) Body weight at weaning. (C) pWAT, iWAT and L weights. (D) Organ index. * $P < 0.05$, ** $P < 0.01$ vs. the control.

index was significantly decreased in the NIC mothers (-48% , Fig. 1D, $P < 0.01$).

3.1.2 Glucose homeostasis and biochemical profiles. Glucose homeostasis and biochemical profiles are shown in Table 2. At the end of lactation, compared with the C mothers, there were no changes in the glycemia and insulin levels in NIC mothers. Concerning insulin sensitivity and β cell function, there was a decreasing trend in the HOMA- β levels (-39% , $P = 0.092$), but there were no changes in the HOMA-IR levels in the NIC mothers, which indicated that prenatal and lactation nicotine exposure could affect the β cell function in mothers. As shown in Table 2, the NIC mothers showed higher

serum TC ($+31\%$, $P < 0.01$), LDL-C ($+56\%$, $P < 0.01$), Castelli index I ($+18\%$, $P < 0.01$) and Castelli index II values ($+33\%$, $P < 0.05$), while the TG, HDL-C and NEFA evaluations were normal.

3.1.3 The metabolic gene expression levels in the liver and adipose tissue. As shown in Fig. 2, compared with the C mothers, the gene expression levels of hepatic lipogenic enzymes (SREBP1c and FAS) were down-regulated (-53% and -55% , $P < 0.05$, respectively), and the ACC and LPL mRNA levels also had decreased trends in the NIC mothers (Fig. 2). Glucose transporter mRNA levels, such as GLUT4, in the liver were also decreased (-72% , $p < 0.05$) in the NIC mothers. Furthermore, SREBP1c, FAS, leptin and GLUT4 mRNA levels were significantly decreased (-48% , -75% , -63% and -65% , Fig. 3, $P < 0.05$, $P < 0.01$, respectively) in the pWAT of the NIC mothers at weaning. These findings suggest that prenatal and lactation nicotine exposure inhibits maternal glucose transport and lipogenesis levels.

Table 2 Glucose homeostasis and biochemical profiles of nicotine-exposed mothers during gestation and lactation ($\bar{x} \pm \text{S.E.M.}$, $n = 8$)

Parameter	Control group	Nicotine group	P Value
Glycemia (mmol L ⁻¹)	6.75 \pm 0.54	6.73 \pm 0.40	0.968
Serum insulin ($\mu\text{g L}^{-1}$)	0.41 \pm 0.04	0.36 \pm 0.07	0.509
HOMA-IR	2.62 \pm 0.20	2.17 \pm 0.13	0.496
HOMA- β	84.00 \pm 15.46	51.04 \pm 17.05	0.092
Total cholesterol (mmol L ⁻¹)	1.66 \pm 0.04	2.18 \pm 0.13	0.002
Triglyceride (mmol L ⁻¹)	0.90 \pm 0.09	0.83 \pm 0.09	0.589
HDL-C (mmol L ⁻¹)	0.75 \pm 0.02	0.83 \pm 0.04	0.132
LDL-C (mmol L ⁻¹)	0.09 \pm 0.01	0.14 \pm 0.01	0.006
NEFA ($\mu\text{mol L}^{-1}$)	662 \pm 116	632 \pm 74	0.824
Castelli index I	2.22 \pm 0.06	2.63 \pm 0.06	0.001
Castelli index II	0.12 \pm 0.01	0.16 \pm 0.01	0.015

3.2 Effects of prenatal and lactation nicotine exposure on pups

3.2.1 Body weight of offspring, sex ratio and litter size. The body weights of the NIC pups during gestation and lactation are shown in Table 3. As is shown, at birth, there were no differences in the pups' body weight between the NIC and C groups. However, compared with the controls, the female and male NIC pups showed higher body weight at weaning ($+8\%$, $P < 0.05$ and $+7\%$, $P < 0.05$, respectively). Additionally, the

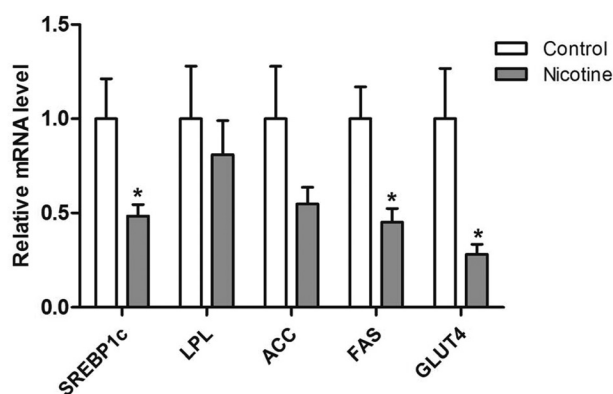


Fig. 2 Metabolic gene expression in the liver of nicotine-exposed mothers during gestation and lactation ($\bar{x} \pm \text{S.E.M.}$, $n = 6-8$). SREBP1c: sterol regulatory element binding protein-1c; LPL: lipoprotein lipase; ACC: acetyl-CoA carboxylase; FAS: fatty acid synthase; GLUT4: glucose transporter 4. * $P < 0.05$ vs. the control.

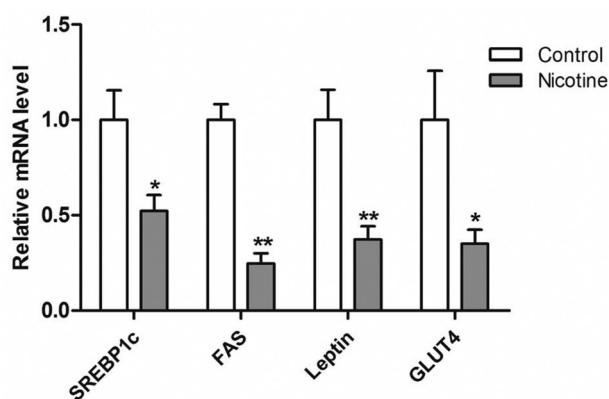


Fig. 3 Metabolic gene expression in the parametrial white adipose tissue (pWAT) of nicotine-exposed mothers during gestation and lactation ($\bar{x} \pm \text{S.E.M.}$, $n = 6-8$). SREBP1c: sterol regulatory element binding protein-1c; FAS: fatty acid synthase; GLUT4: glucose transporter type 4. * $P < 0.05$, ** $P < 0.01$ vs. the control.

Table 3 Body weight, sex ratio and litter size of nicotine-exposed pups during gestation and lactation at weaning ($\bar{x} \pm \text{S.E.M.}$, $n = 8$)

Parameter	Control group	Nicotine group	P Value
Litter size	13.25 \pm 0.45	13.88 \pm 0.58	0.410
Sex ratio (proportion of females per litter, %)	47.52 \pm 5.86	48.69 \pm 4.36	0.875
Body weight of female (at birth, litter)	5.98 \pm 0.17	6.00 \pm 0.12	0.931
Body weight of male (at birth, litter)	6.29 \pm 0.18	6.28 \pm 0.15	0.952
Body weight of female (at weaning)	71.28 \pm 1.89	77.26 \pm 1.17	0.018
Body weight of male (at weaning)	81.04 \pm 1.73	86.43 \pm 1.33	0.027

number of pups per litter was not statistically different between the groups (Table 3). We also observed that the sex ratio of newborn pups was not altered by prenatal nicotine exposure: the proportions of females per litter were similar in C (47.52 ± 5.86) and in NIC pups (48.69 ± 4.36 ; $P = 0.87$) (Table 3).

3.2.2 Glucose homeostasis and biochemical profiles. The glucose homeostasis and blood biochemical levels from the NIC pups during gestation and lactation are depicted in Table 4. In this study, the insulin levels in all of the groups were too low to detect. Compared with the C pups, there were no changes in the glycemia and leptin levels in the weaned female NIC pups. The weaned male NIC pups had decreased trends for the glycemia level (-11% , $P = 0.079$); however, the leptin levels were unchanged between the groups. Regarding the lipid profiles of the pups, both the female and male NIC pups presented higher serum TC levels ($+25\%$, $P < 0.05$; $+19\%$, $P < 0.05$) while their TG, LDL-C and NEFA levels were unchanged. In the weaned female NIC pups, there was an increasing trend for HDL-C ($+25\%$, $P = 0.080$) and Castelli index I ($+9\%$, $P = 0.097$). The weaned male NIC pups showed higher Castelli index I ($+10\%$, $P < 0.01$) values; however, their Castelli index II values and HDL-C were unchanged.

3.2.3 The metabolic gene expression levels in the liver and adipose tissue. Compared with the controls, in the female NIC pups at weaning, the hepatic FAS and ACC gene expression levels presented a decreasing trend (-33% , $P = 0.085$ and -36% , $P = 0.077$) while the GLUT4 mRNA levels remained unchanged (Fig. 4A). The FAS, leptin and GLUT4 mRNA levels in the pWAT from the female NIC pups at weaning remained unchanged (Fig. 4B). However, in the male NIC pups at weaning, the FAS mRNA levels were significantly decreased (-11% , $P < 0.05$), while there were no effects on the ACC and GLUT4 expression levels in the liver (Fig. 5A). Additionally, the FAS mRNA levels were significantly increased by 1.86 ± 0.40 -fold ($P < 0.05$); however, there were no effects on the leptin and GLUT4 expression levels in the eWAT (Fig. 5B). These findings suggest that prenatal and lactation nicotine exposure lowers lipid synthesis in the liver and adipose tissue of female pups; however, in male NIC pups, lipid synthesis was enhanced in the adipose tissue but reduced in the liver.

4 Discussion

In this present study, we used an experimental model of prenatal and lactation nicotine exposure ($2.0 \text{ mg kg}^{-1} \text{ d}^{-1}$) to show that maternal nicotine exposure leads to important changes in glucose homeostasis, lipogenesis and lipid metabolic profiles in both the mother and offspring. The dose ($1 \text{ mg kg}^{-1} \text{ d}^{-1}$) of nicotine has been shown to lead to cotinine levels in maternal serum that are similar to those observed in moderate female smokers ($80-163 \text{ ng ml}^{-1}$).⁴⁸ Somm *et al.* reported that the circulating nicotine metabolite levels in gestating females were found to range between 250 and 300 ng ml^{-1} under nicotine

Table 4 Glucose homeostasis and biochemical profiles of nicotine-exposed pups during gestation and lactation at weaning ($\bar{x} \pm \text{S.E.M.}$, $n = 8$)

Parameter	Female		Male	
	Control	Nicotine	Control	Nicotine
Glycemia (mmol L^{-1})	4.19 ± 0.08	4.32 ± 0.21	4.37 ± 0.20	3.90 ± 0.35
Serum leptin ($\mu\text{g L}^{-1}$)	3.48 ± 0.09	3.47 ± 0.12	3.23 ± 0.12	3.51 ± 0.25
Total cholesterol (mmol L^{-1})	1.90 ± 0.10	$2.38 \pm 0.16^*$	1.93 ± 0.11	$2.29 \pm 0.11^*$
Triglyceride (mmol L^{-1})	1.90 ± 0.32	1.86 ± 0.18	1.61 ± 0.17	1.86 ± 0.20
HDL-C (mmol L^{-1})	0.72 ± 0.04	0.82 ± 0.04	0.70 ± 0.04	0.75 ± 0.04
LDL-C (mmol L^{-1})	0.30 ± 0.03	0.29 ± 0.02	0.27 ± 0.02	0.31 ± 0.02
NEFA ($\mu\text{mol L}^{-1}$)	1060 ± 126	950 ± 52	877 ± 64	876 ± 64
Castelli index I	2.66 ± 0.07	2.89 ± 0.10	2.77 ± 0.07	$3.05 \pm 0.05^{**}$
Castelli index II	0.42 ± 0.04	0.36 ± 0.02	0.40 ± 0.03	0.42 ± 0.03

* $P < 0.05$, ** $P < 0.01$ vs. the control.

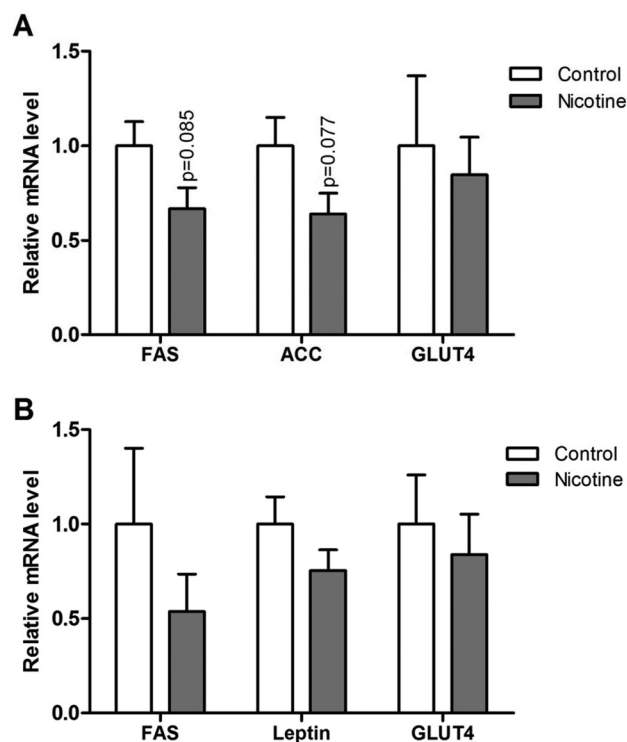


Fig. 4 Metabolic gene expression in nicotine-exposed female pups during gestation and lactation at weaning ($\bar{x} \pm \text{S.E.M.}$, $n = 6-8$). (A) Liver; (B) parametrial white adipose tissue (pWAT). FAS: fatty acid synthase; ACC: acetyl-CoA carboxylase; GLUT4: glucose transporter 4.

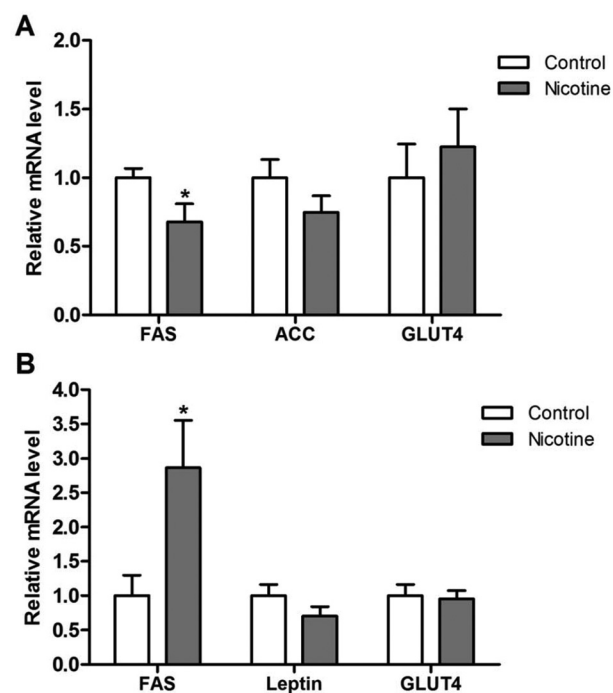


Fig. 5 Metabolic gene expression in nicotine-exposed male pups during gestation and lactation at weaning ($\bar{x} \pm \text{S.E.M.}$, $n = 6-8$). (A) Liver; (B) epididymal white adipose tissue (eWAT). FAS: fatty acid synthase; ACC: acetyl-CoA carboxylase; GLUT4: glucose transporter 4. * $P < 0.05$ vs. the control.

exposure ($3 \text{ mg kg}^{-1} \text{ d}^{-1}$).⁵⁰ These values consistent with those found in smokers consuming between 10 and 19 cigarettes per day whose serum cotinine levels were between 320 and 430 ng ml^{-1} .⁵¹ Typically, plasma nicotine levels in female heavy smokers were found to range between 0.3 and 0.6 μM .⁴⁹ Therefore, we used this dose of nicotine ($2.0 \text{ mg kg}^{-1} \text{ d}^{-1}$), which is intended to be a model of mothers who are moderate to heavy cigarette smokers, to investigate the association between maternal nicotine exposure and fetal glucose homeostasis, lipogenesis and lipid metabolic profiles.

4.1 Changes in mothers

It was reported that adult rats treated with nicotine lost weight and were hypophagic;⁵⁵ however, Chen *et al.* observed that treating pregnant rats with nicotine did not affect maternal body weight or food intake.⁵⁶ Oliveira *et al.* also found that nicotine exposure during lactation produced no change in the mothers' body weight and food intake.²¹ In our study, in the model of maternal nicotine exposure during pregnancy and lactation, we observed that the NIC mothers lost total body, pWAT and iWAT weights. We also found that prenatal and lactation nicotine exposure inhibited the mothers' glucose

transport and lipogenesis through a decrease of metabolic gene expression in the liver and adipose tissue. These results indicated that a decrease in body weight in the NIC mothers might be associated with decreased glucose transport and lipogenesis. Indeed, it was shown that nicotine can lower the set points of body weight in both rodents⁵⁷ and humans.⁵⁸ Studies have shown that acute and chronic nicotine treatments result in a decrease in body weight of rodents, which was associated with one or more of the following effects:^{59,60} (a) decreased food intake due to reduced meal size (*i.e.*, increased satiation),⁶¹ (b) increased energy metabolism,⁶² (c) increased lipolysis,⁶³ or (d) increased physical activity.^{59,60}

Regarding glucose homeostasis, in our study, the NIC mothers showed normoglycemia. Normoglycemia was described in maternal smoking during pregnancy, which was associated with lower liver glycogen content and glucose intolerance.⁶⁴ Santos-Silva *et al.* also reported that maternal smoking during lactation exhibited no change in blood glucose levels.⁴³ Furthermore, Santos-Silva *et al.* found that maternal smoking during lactation presented hypoinsulinemia and lower HOMA-IR levels, suggesting higher insulin sensitivity. In our study, we observed a decreased HOMA- β level trend in the NIC mothers, which indicated that prenatal and lactation nicotine exposure could affect β cell function in mothers. Recent evidence has indicated that smoking is an independent risk factor for acute pancreatitis.⁶⁵ Epidemiological evidence strongly suggests an association between cigarette smoking and pancreatic injury and that nicotine can induce impairment in glucose homeostasis, as well as pancreatic islet cell apoptosis and damage.⁶⁶ Furthermore, mRNA levels of GLUT4 in the liver and pWAT were also significantly decreased in the observed nicotine-exposed mothers at weaning. Taken together, these studies show that in rats, prenatal and lactation nicotine exposure can affect maternal pancreatic function and glucose transport, suggesting a risk for the future development of hyperglycemia in NIC mothers. In addition, Wang *et al.* also showed that skeletal muscle accounts for 70–80% of the insulin-stimulated glucose uptake, exerting a key role in regulating whole-body glucose homeostasis.⁶⁷ Therefore, it is possible that GLUT-4 in muscle could be a compensatory mechanism for normoglycemia in NIC mothers. The mechanism still calls for further research and confirmation.

It was reported that cigarette smoking contributes to cardiovascular disease through lipid profile alterations.^{68,69} Different results have been reported for maternal lipid profile alterations because of nicotine treatment. Santos-Silva *et al.* reported that smoking exposed-mothers during lactation exhibited lower TG and VLDL-C levels.⁴³ Oliveira *et al.* showed that nicotine exposed-mothers during lactation presented higher serum HDL-C levels.²¹ In our study, in prenatal and lactation nicotine exposed-mothers, we observed higher LDL-C levels and hypercholesterolemia, as well as higher Castelli index values at weaning, which indicated a high risk for dyslipidemia and atherogenesis development in the mothers. However, metabolic gene expression levels in the liver and adipose tissue (*e.g.* SREBP1c, FAS and leptin) were down-regulated, which indi-

cated decreased lipogenesis in the weaned NIC mothers. Taken together, these findings suggest that prenatal and lactation nicotine exposure slows down lipogenesis in the liver and adipose tissue in mothers, which may account for the observed decreased body weights and also may be a compensatory mechanism that leads to higher LDL-C levels as well as hypercholesterolemia.

4.2 Changes in the pups

In previous experimental studies, pre- and post-natal nicotine exposure failed to cause changes in the body weight of pups during the exposure period.^{41,42} Santos-Silva *et al.* found that there were no body weight alterations in pups whose mothers were smoke-exposed during lactation.⁴³ Oliveira *et al.* demonstrated that maternal nicotine exposure did not affect the body weight gain of NIC offspring during lactation.⁴⁰ In this study, in the model of maternal exposure to NIC during pregnancy and lactation, we also found that there were no differences in the pups' birth weight between two groups. However, the weaned female and male NIC pups showed higher body weight. Higher total body fat mass was also described in suckling pups whose mothers were exposed to nicotine during lactation.⁴⁰ Nicotine exposure from gestation to the 10th day of lactation increased the body weight in offspring at 35 days of age.⁴¹ Through the analysis of these studies, the discrepancy in body weight of offspring after maternal nicotine exposure may involve strains of animals and different doses, routes, and windows of exposure.^{40,42,50} In addition, this discrepancy may result from the effect of other components in the tobacco used in some studies. Furthermore, Oliveira *et al.* have demonstrated that maternal nicotine exposure during lactation programs for a higher body weight gain and adiposity in adult life of the offspring as well as for hyperleptinemia although lipid profile was not changed in adulthood.⁴⁰ Nicotine exposure during lactation possibly leads to a neonatal thyroid hypofunction and programs for central hypothyroidism, which may partially explain the overweight status in adulthood.⁴⁰ Rats whose mothers were treated with nicotine for 14 days before mating and during pregnancy until weaning were heavier at 70 days of age compared with controls and had higher body weight and visceral adipose tissue levels at 180 days of age.⁴²

As previously mentioned, smoking or nicotine exposure is related to the development of insulin resistance and type 2 diabetes.^{70,71} In this study, weaned female NIC pups showed no alteration in glycemia, while weaned male NIC pups showed slightly lower glycemia. Additionally, there was no effect on GLUT4 expression in the liver and adipose tissue in the weaned female and male NIC pups. These results indicated that prenatal and lactation nicotine exposure nearly did not affect glucose homeostasis in the offspring and only decreased glucose levels in the male offspring. The effects on glucose homeostasis in the offspring were weaker than those in a report by Santos-Silva *et al.*, who found that smoke-exposed pups during lactation showed lower glycemia and hyperinsulinemia levels and higher HOMA- β levels.⁴³ This difference may

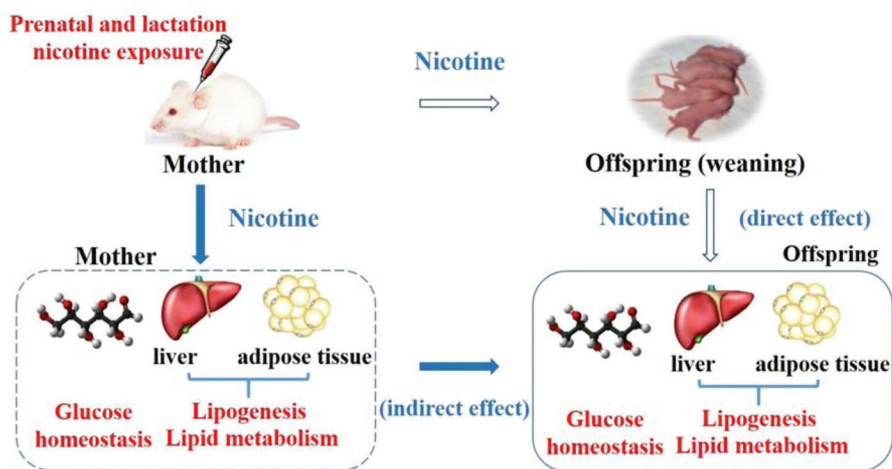


Fig. 6 Direct and indirect effects of maternal nicotine exposure on pups' glucose homeostasis, lipogenesis and lipid metabolism.

arise from the synergistic effect of other components in the tobacco used in Santos-Silva's study.

Concerning the pups' lipid profiles, both the female and male NIC pups presented higher serum TC levels at weaning. Additionally, there were increased HDL-C and Castelli index I trends in the weaned female NIC pups, and the weaned male NIC pups had higher Castelli index I values, which indicated a high dyslipidemia development risk in the offspring. Santos-Silva *et al.* showed that pups whose mothers were smoke-exposed during lactation presented higher serum TG and HDL-C levels as well as lower LDL-C levels.⁴³ Oliveira *et al.* detected higher serum HDL-C levels in 15-day-old NIC pups whose mothers were exposed to nicotine during lactation.²¹ Ma *et al.* showed that nicotine exposure during pregnancy and lactation led to an increase in circulating and hepatic TG levels in adult male offspring.¹¹ Additionally, in our study, the leptin levels were unchanged in weaned female and male NIC pups. Santos-Silva showed that weaned smoke-exposed pups showed no leptin level changes.⁴³ Oliveira *et al.* observed that 15-day-old NIC pups whose mothers were exposed to nicotine during lactation presented higher serum leptin levels.²¹ Furthermore, in our study, prenatal and lactation nicotine exposure affected lipogenesis gene expression levels in the liver and adipose tissue, and there are also gender-specific effects. FAS expressions in the liver and adipose tissue were down-regulated in the weaned female NIC pups. However, in the weaned male NIC pups, FAS expression was down-regulated in the liver but up-regulated in the adipose tissue. These results indicated that maternal nicotine exposure during pregnancy and lactation affects early adipogenesis and lipogenesis in pups, and FAS may play a vital role in early lipogenesis, which may promote several important metabolic disorders in the progeny.

4.3 Differences between mothers and pups

In this study, in the model of maternal nicotine exposure during pregnancy and lactation, the NIC mothers had lower

total body weight, WAT weight and HOMA- β levels, higher serum TC, LDL-C, and Castelli index values, and lower metabolic gene expression levels in the liver and adipose tissue. However, the weaned female and male NIC pups presented higher body weights and serum TC levels. The weaned female NIC pups presented increased HDL-C and Castelli index I trends. The weaned male NIC pups showed lower glycemia and Castelli index I levels. Additionally, the weaned female NIC pups presented lower FAS gene expression levels in the liver and adipose tissue, while the weaned male NIC pups presented lower hepatic FAS expression and higher adipose FAS expression. From these results, we found some characteristic trends: (1) the effects of prenatal and lactation nicotine exposure on mothers were different from those on their offspring; (2) the effects of prenatal and lactation nicotine exposure on lipogenesis and lipid metabolism were stronger than those on glucose homeostasis in the mothers and offspring; (3) our findings may result from functional changes in both the mothers and pups, which agrees with Oliveira's findings;²¹ and (4) concerning the pups, on the one hand, nicotine transfer through the placenta or maternal milk may lead to direct roles in the offspring. On the other hand, maternal alterations caused by nicotine treatment, such as glucose homeostasis and/or lipogenesis and lipid metabolism, may indirectly have an influence on the offspring (Fig. 6). (5) There are gender-specific effects on pups, and the effects on male offspring were more obvious than those on the females.

5. Conclusions

In conclusion, we have demonstrated that prenatal and lactation nicotine exposure induced deleterious effects on glucose homeostasis, lipogenesis and lipid metabolism in both mothers and pups, which may promote several important metabolic disorders in the progeny. The effects on pups include the direct roles of nicotine and indirect roles of

maternal alterations. Additionally, there are gender-specific effects on pups and the effects on male offspring were more obvious than those on the females. Therefore, the implication of these results is that pregnant women and women who are breastfeeding should avoid exposure to tobacco smoke or nicotine.

Conflict of interest

There are no conflicts of interest to declare.

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